Asian Journal of Chemistry; Vol. 23, No. 6 (2011), 2407-2410

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

High Performance Liquid Chromatographic Determination of Solasodine in *Solanum* Species

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(*Received*: 26 April 2010; *Accepted*: 4 February 2011) AJC-9579

This study incorporates HPLC analysis of solasodine from *Solanum indicum* Linn. and *Solanum trilobatum* Linn. Solasodine was determined by HPLC using SphereClone 3µ ODS, 100 × 4.6 mm as an analytical column with the mobile phase 10 mM phosphate buffer-acetonitrile (75:25, v/v) adjusted to pH 3.0. The flow rate was adjusted to 1.0 mL min^{-1}. The injection volume was adjusted to 50 µL and the absorption was made at 530 nm. Linear calibration curve was obtained over the concentration range of 2.5-60.0 µg mL^{-1} of solasodine with a correlation coefficient of 0.9980. The limit of detection (LOD) was found to be 0.13 µg mL^{-1}. The limit of quantification (LOQ) value was found to be 0.43 µg mL^{-1}. The RSD of the proposed method obtained by assaying six replicate injections of 10, 20 and 30 µg mL^{-1} of solasodine for intra-day and inter-day were found to be 1.8-2.4 and 1.0-2.8 %, respectively. The mean percentage recovery was found to be 97.23 ± 4.71 %. The proposed method was applied to the determination of solasodine in *Solanum indicum* Linn. and *Solanum trilobatum* Linn. in Solanaceae family. The mean contents of solasodine in *Solanum indicum* Linn. and *Solanum trilobatum* Linn. were found to be 0.48 and 1.32 mg g^{-1}, respectively. The method has been applied to the determination of solasodine in various plant samples.

Key Words: Solasodine, High performance liquid chromatography, Ion-pair complex.

INTRODUCTION

Solasodine, a spiroketal alkaloid sapogenin with a hetorocyclic nitrogen atom (Fig. 1), is used for the production of steroid drug in medical industry. It is also used in the preparation of contraceptive drug. Solasodine is present in a number of *Solanum* species (Solanaceae) such as *S. khasianum*, *S. xanthocarpum*, *S. nigrum*, *S. gracile*, *S. laciniatum*, *etc.*[1]. A number of traditional herbs containing solasodine have been used in the Indian system of medicine[2-5]. Solasodine have been reported to provide anticancer[5], insecticidal[6], antiaccelerator cardiac activities[7] and antioxidant activities[8]. A number of analytical methods such as high performance thin layer chromatography[9-11], high performance liquid chromatography[12-20], capillary electrophoresis[21-23], gas chromatography[24-27] and colorimetric method[28-35] are available for determination of solasodine. Solasodine does not have a conjugated double bond in its structure.

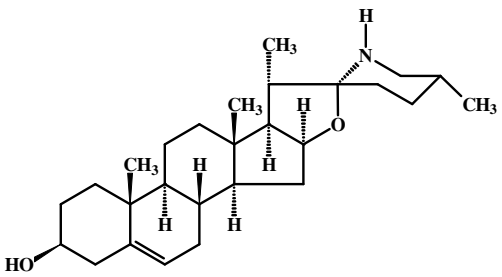

Fig. 1. Structure of solasodine

The nitrogen is protonated and forms complexes that are extractable into organic solvent like chloroform. Liquid-liquid extraction is one of the most versatile techniques for sample matrix separation. It has been applied to various analytical fields. However, manual extractions present a series of drawbacks such as high consumption of sample and toxic organic solvents, low sampling frequency and loss of analyte through manipulation. In this studies the use of high performance liquid chromatography (HPLC) has been recognized as a developed method for the separation and quantitative analysis of solasodine. Because solasodine lacks conjugate double bonds in its structure, detection of solasodine in the ultraviolet range is not fully convincing. It would be more appropriate if the λ_{max} of solasodine could be enhancing to ensure a more effective detection. Hence, an attempt was made to introduce an ion-pair complexation prior to HPLC analysis of solasodine.

EXPERIMENTAL

All the reagents used were analytical reagent grade. Deionized water of milli-Q water purification system, (Millipore Co, USA) was used throughout the experiments. Solasodine reference standard of 99 % purity was purchased from Sigma (No. 204-774-2, Sigma Chemical Co., USA).

Standard solasodine solution was prepared in chloroform. Ion-pair complexation was done by the method of Trivedi *et al.*[10]. The standard solution was derivatized by treated with 0.04 % solution of methyl orange. The ion-par complex was extracted with chloroform, dried over anhydrous sodium sulfate and added with 10 mL of 0.5 M hydrochloric acid. A standard aqueous acid layer was concentrated to 1 mL on a water bath. The solution was transferred to a 10 mL volumetric flask and diluted to the mark with methanol.

Stock solution of methyl orange (0.04 %, w/v) was freshly prepared daily by dissolving 0.04 g of methyl orange in 100 mL of deionized water and kept in a suitable reagent bottle.

Sample preparation: The steroids based of the solasodine group occur naturally as the glycoside usually containing three sugars. The hydrolysis of glycoside yield the steroid alkaloid in the aglycone forms for example, the glycoside solanine, solasodine and glucose, galactose and rhamnose. Alkaloid content of this material is usually determined by extraction of the dried material using continuous extraction apparatus, removal of the solvent and precipitation of the bases, followed by dissolving in acid. About 1 g of each dried fruit (previously dried at 50 ºC for 4 h) was accurately weighed and transferred into a clean mortar. The sample was ground and 20 mL of 95 % ethanol was added and mixed thoroughly, then transferred into a-250 mL beaker. The solution was heated in a water bath at 70 ºC for 0.5 h and then filtered through Whatman No. 1 filter paper into a-50 mL volumetric flask, followed by washing with several portions of 95 % ethanol. Each solution was adjusted to 50 mL with 95 % ethanol. Aliquot of 5.0 mL of ethanolic extract from each sample was transferred into 20 mL test tube. The ethanol was removed ethanol by holding the tubes at 70 ºC while gently blowing an air current into each tube. To each tube, 3.0 mL of 1.0 mol L^{-1} hydrochloric acid was added and the temperature of water bath was increased to 100 ºC. The tube containing each acidified sample solution was then held at 100 ºC for 2 h. The acidic sample solution was neutralized by adding 3.0 mL of 1.0 mol L^{-1} sodium hydroxide followed by addition of 2.0 mL glacial acetic acid into each tube. Each solution was transferred into a 25 mL volumetric flask and then diluted to the mark with distilled water. The sample solution (10 mL) was derivatized by treated with 0.04 % solution of methyl orange. The ion-par complex was extracted with chloroform, dried over anhydrous sodium sulfate and added with 10 mL of 0.5 M hydrochloric acid. The aqueous acid layer was concentrated to 1 mL on a water bath. The solution was transferred to a 10 mL volumetric flask and diluted to the mark with methanol.

The chromatographic system for the separation and analysis of solasodine in various *Solanum* species was carried out in the isocratic mode. Ion-pair complexation was formed as described for solasodine standard solution and HPLC was carried out. The HPLC column used was SphereClone 3µODS (100 × 4.6 mm, 3 µm) and the mobile phase was 10 mM phosphate buffer-acetonitrile (75:25) pH 3.0. The flow rate was adjusted to 1.0 mL min^{-1}. The injection volume was adjusted to 50 mL and the absorption was made at 530 nm.

RESULTS AND DISCUSSION

In this investigation, various mobile phase compositions and concentrations were studied. Well-defined peaks were observed at phosphate buffer pH 3.0. Different concentrations and type of organic modifiers (methanol or acetonitrile) were then tried and a good separation of analytes in a short analysis time was achieved by using acetonitrile as organic modifier. The high performance liquid chromatographic method involving pre-chromatographic derivatization step for the determination of solasodine was done by forming an ion-pair complex of the heterocyclic nitrogen using the acidic dye methyl orange. The method was validated and applied to the determination of solasodine content in *Solanum indicum* Linn. and *Solanum trilobatum* Linn.

Selection of the chromatographic conditions: The aim of the present work is to develop a simple, rapid and specific HPLC method for the analysis of solasodine. A preliminary experiment was carried out to investigate the spectral characteristics of the above complex. The hydrolysis product of solasodine aglycone was complexed with methyl orange solution which was then extracted into chloroform yielding a yellow coloured complex. The absorption spectrum of such a complex was studied by batch wise spectrophotometrically. The absorption spectrum of ion-pairing solasodine-methyl orange complex in chloroform was obtained by scanning the wavelength over the range of 200-700 nm. It was found that the yellow colour of solasodine-methyl orange complex formed which showed maximum absorption at 530 nm.

The best separation was obtained using a reverse phase SphereClone 3µODS column at room temperature, with a mobile phase consisted of 10 mM phosphate buffer-acetonitrile (75:25) pH 3.0. The flow rate was 1.0 mL min^{-1} and the effluent was monitor at 530 nm. Under the optimum conditions adopted, the analyte was fully separated in 10 min with symmetrical peak. The chromatogram of solasodine is presented in Fig. 2.

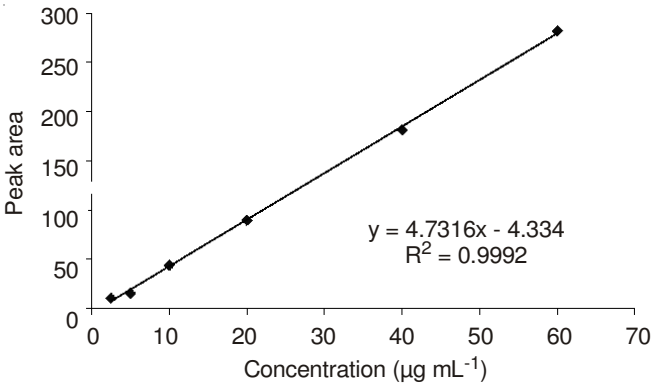

Fig. 2. Linear calibration curve of solasodine

To test the suitability of the proposed HPLC system and to validate its performance characteristics such as linearity, limit of detection, limit of quantitation, precision and accuracy were performed using solasodine under the selected conditions.

Linearity of calibration graph: The linearity of responses to solasodine was determined. Calibration curve was obtained by using the least-square linear regression analysis of the studied solasodine peak area (y) *versus* analyte concentration (x). Each concentration was tested in triplicate. Linear calibration curve was obtained over the concentration range of 2.5-60 μg mL^{-1} of solasodine. The standard solution was injected into the HPLC system. Linear calibration graph over the concentration range 2.5-60 μg mL^{-1} of solasodine was obtained with a correlation coefficient of 0.9992.

Limit of detection and limit of quantification: The detection limit of the method was investigated by injecting standard solution of solasodine into the HPLC column. The limit of detection (LOD) was found to be 0.13 μg mL^{-1}. It is calculated as three times of the standard deviation. The limit of quantification (LOQ) value was found to be 0.43 mg mL^{-1}. This value was calculated directly from the calibration graph. It is defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision, LOQ may be expressed as: LOQ = 10SD/S where S is the slope of calibration graph and SD is standard deviation.

Precision and accuracy: The precision of the method was determined by measuring the repeatability (intraday precision) and the intermediate precision (interday precision), both expressed as relative standard deviation (RSD). The precision was evaluated by assaying six replicate injections of 10, 20 and 30 μg mL^{-1} of solasodine. The repeatability was evaluated each sample on the same day under the same experimental conditions, 2.4, 2.0 and 1.8 %, respectively. The intermediate precision was evaluated by assaying each sample on three different days, 2.0, 2.8 and 1.0 %, respectively. The results are illustrated in Table-1.

The recoveries were determined by using standard addition method. Solasodine standard concentration of 10, 20 and 30 μg mL^{-1} were added and mixed with known aliquots of sample solutions, the sample was extracted and analyzed using the proposed method. The percentage recoveries of the spiked solasodine in *Solanum indicum* Linn. and *Solanum trilobatum* Linn. solution were found to be 98.80-103.00 and 96.80-102.20 %, respectively. Results are presented in Table-2.

TABLE-1
INTER-DAY AND INTRA-DAY PRECISION STUDIES OF SOLASODINE

Solasodine added (μg mL^{-1})	Intra-day precision*		Inter-day precision*	
	Found ± SD	% RSD	Found ± SD	% RSD
10	10.48 ± 0.2	2.4	10.52 ± 0.4	2.0
20	20.10 ± 0.4	2.0	20.04 ± 0.6	2.8
30	30.80 ± 0.2	1.8	30.10 ± 0.2	1.0

*Average of three determinations.

TABLE-2
ACCURACY DATA OF SOLASODINE USING STANDARD ADDITION METHOD

Sample name	Solasodine concentration*		Recovery* (%)
	Added (μg mL^{-1})	Found (μg mL^{-1} ± SD)	
Solanum indicum Linn.	10	9.96 ± 0.02	98.80
	20	20.18 ± 2.10	103.00
	30	29.92 ± 0.80	99.20
Solanum trilobatum Linn.	10	9.84 ± 1.00	96.80
	20	19.98 ± 1.80	99.80
	30	30.32 ± 0.50	102.20

*Average of three determinations.

Application: The proposed LC method was applied to the determination of solasodine in *Solanum indicum* Linn. and *Solanum trilobatum* Linn. in Solanaceae family. The extracts of *Solanum* were determined using the sample preparation steps and the optimum LC conditions. Solasodine contents in *Solanum* samples were presented in Table-3. The chromatograms of standard and sample solutions are shown in Figs. 3 and 4, respectively.

TABLE-3
SOLASODINE CONTENT IN *Solanum* SAMPLES

Sample name	Solasodine*	
	Found (μg mL^{-1}) (mean ± SD)	Concentration (mg g^{-1})
Solanum indicum Linn.	9.69 ± 0.62	0.48
Solanum trilobatum Linn.	26.99 ± 1.09	1.32

*Average of three determinations.

Conclusion

The developed LC system is precise, selective and accurate for determination of solasodine in *Solanum* species. Thus this method involves a simple extraction-hydrolysis step, selective

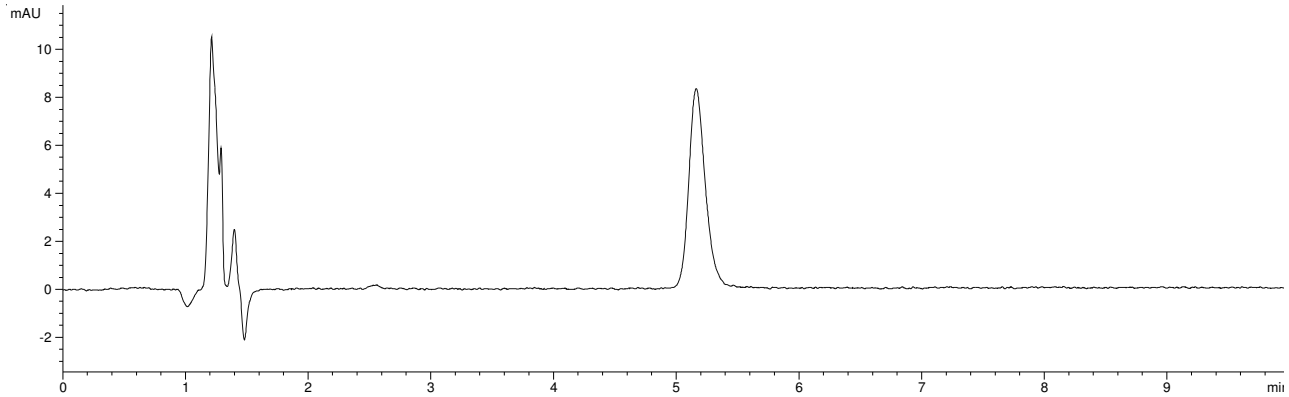

Fig. 3. Chromatogram of solasodine standard (at concentration 2.5 μg mL^{-1})

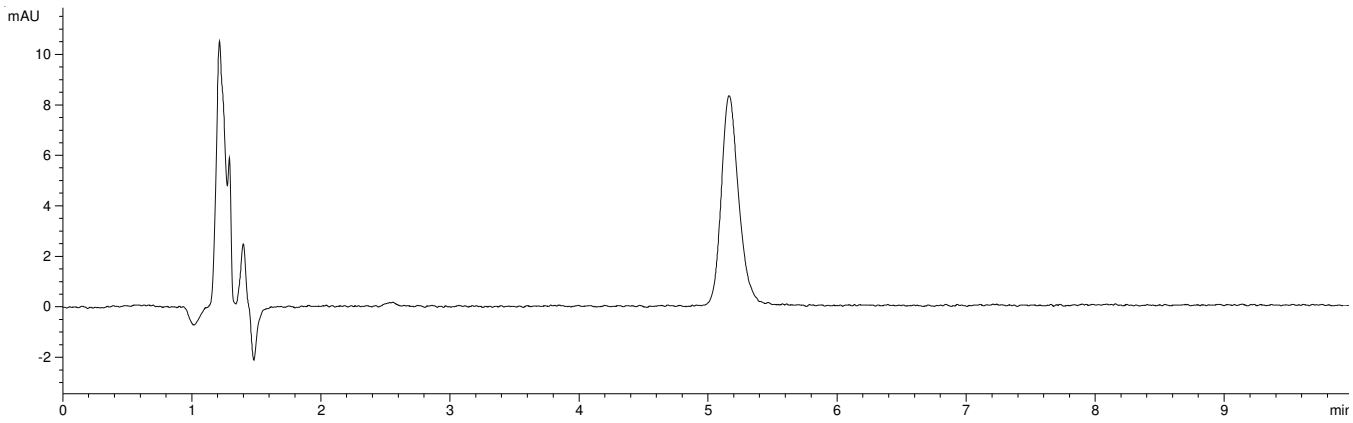

Fig. 4. Chromatogram of solasodine in *Solanum indicum* Linn

extraction of the solasodine and quantification of the yellow coloured complex at its λ_{max} 530 nm. The proposed LC method could be used for the analysis of similar nitrogen-containing alkaloids. For *Solanum* samples, it is important to hydrolyze glycosides which can be reacted with methyl orange to form yellow coloured complexes.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the Department of Chemistry, Faculty of Science and Technology, Pibulsongkham Rajabhat University for partial support and providing the research facilities.

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